Research Article

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Impact of the goldspotted oak borer, *Agrilus auroguttatus*, on the health of coast live oak before and after treatment with two systemic insecticides

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Abstract

BACKGROUND: The invasive goldspotted oak borer, *Agrilus auroguttatus*, is threatening the health and survival of oak trees in San Diego County, California. From two sites in the core area of the infestation, we report a 2.5 year investigation of the impact of *A. auroguttatus* on coast live oak, *Quercus agrifolia*, before and after treatment with two systemic insecticides, emamectin benzoate (EB) and imidacloprid (IC).

RESULTS: None of the 446 survey trees died during the study. The crown dieback rating of most trees at both study sites remained unchanged, regardless of insecticide treatment. A higher cumulative increase in the number of *A. auroguttatus* emergence holes was observed on trees that were previously infested and on trees with larger diameters. Over the 2.5 year period, the new infestation rates of initially uninfested trees across the untreated and treated groups were 50% (EB) and 32% (IC), and neither EB nor IC treatment affected cumulative increases in the number of emergence holes. EB-injected trees did not have significant annual increases in the number of *A. auroguttatus* emergence holes at either 1.5 or 2.5 years compared with that at 0.5 years, whereas untreated trees had significant annual increases. Although IC-injected trees had a significantly greater annual increment in the number of emergence holes than untreated trees during the last year of the study, treated trees had significant reductions in annual increases in emergence holes at both 1.5 and 2.5 years compared with that at 0.5 years. Untreated trees had no significant reduction in the annual increase in emergence holes at 1.5 and 2.5 years.

CONCLUSIONS: *A. auroguttatus* preferentially attacked previously infested and larger (diameter at breast height > 15–30 cm) oak trees, but the attacks led to very gradual changes in the health of the trees. Both EB and IC provided minor suppressive effects on *A. auroguttatus* emergence.

Keywords: Buprestidae; Coleoptera; emamectin benzoate; imidacloprid; invasive species; *Quercus agrifolia*

1 INTRODUCTION

The goldspotted oak borer, *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae), has killed nearly 28 000 oak trees (*Quercus* spp.) in eastern San Diego County, California, since 2002.[1] The beetle continues to spread through flight dispersal in San Diego County (Fig. 1),[2] was detected in neighboring Riverside County in November 2012[3] and is likely to invade other areas of California via movement of infested firewood. In the introduced range in California, oak mortality caused by *A. auroguttatus* has been observed more frequently with coast live oak, *Q. agrifolia* Née, and California black oak, *Q. kelloggii* Newb. (both classified as red oaks in the family Fagaceae and section *Lobatae*), and with canyon live oak, *Q. chrysolepis* Liebm. (a taxonomically intermediate species in section *Protobalanus*), but rarely with Engelmann oak, *Q. engelmannii* Greene (a white oak in section *Quercus*).[4–7] In the native range (southeastern Arizona), *A. auroguttatus* also prefers to colonize red oak species.[5–7]

Although adults of *Agrilus* spp. feed on foliage,[8,9] this damage does not appear to have a major impact on tree health.[10] Feeding by *A. auroguttatus* larvae at the phloem/xylem interface of the lower portion of the main stem is the major damage caused by this insect to oaks.[11]

Early detection[2] and assessment of infestation level[11] are critical tools for focusing management programs. Currently, the primary

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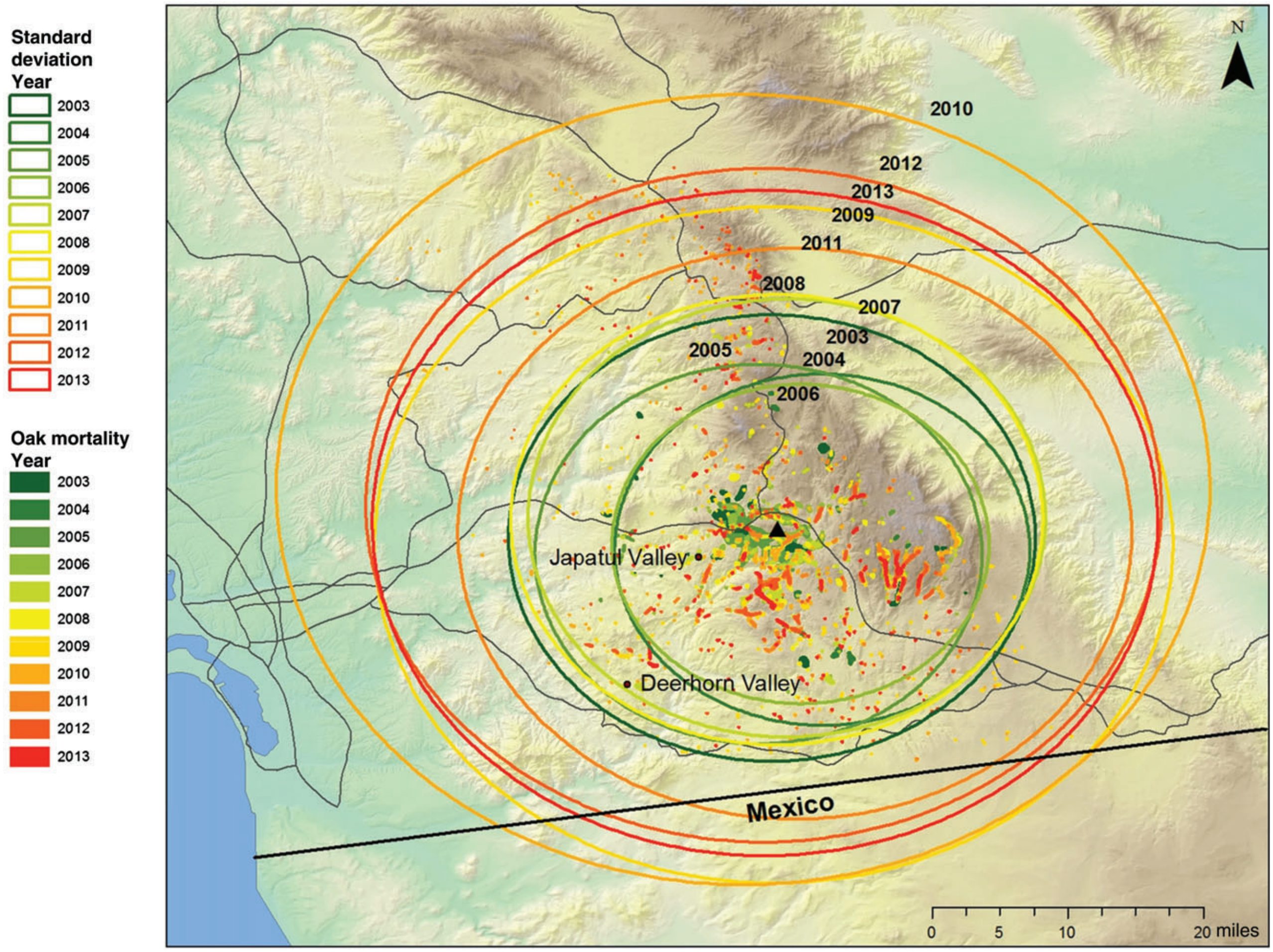

Figure 1. Location of research sites in southern California (San Diego County) relative to the distribution of oak mortality documented by aerial sketch mapping.[1] Large circles characterize the area potentially affected by *Agrilus auroguttatus* by year. Both research sites were located in the core area of the zone of infestation.

approach available for managing *A. auroguttatus* involves preventing infestation of healthy trees by limiting movement of and destroying insects in infested wood through procedures such as debarking, tarping or grinding.[3,10,12] Trees targeted for removal and sanitation are the so-called 'brood' trees, which appear to concentrate a large proportion of local populations in newly infested areas.[13] In addition to these sanitation measures, biological control has been considered as a potential management technique.[14] The following biological control agents for *A. auroguttatus* have been identified: an egg parasitoid, *Trichogramma* sp. (Hymenoptera: Trichogrammidae); larval parasitoids, *Atanycolus simplex* (Cresson) (Hymenoptera: Braconidae) and *Calosota elongata* Gibson (Hymenoptera: Eupelmidae); a predatory mite, *Pyemotes tritici* (LaGrèze-Fossat & Montagné) (Trombidiformes: Pyemotidae).[5,14–18] Woodpeckers (Aves: Piciformes: Picidae) are also known predators of *A. auroguttatus* larvae and pupae, but the extent to which they regulate population density has yet to be assessed.[10,11]

There are no published studies of the efficacy of insecticide treatments for *A. auroguttatus* because it was never considered to be an economic or ecological threat in its native region and only recently has been recognized as a threat in the introduced region.[10,19] Options used for similar insect pest species include bark and foliar applications of contact insecticides such as carbamates (carbaryl) or pyrethroids that target first-instar larvae or adults, or systemic insecticides applied as soil injections or drenches, or trunk injections or sprays that target larvae beneath the bark. In urban and suburban areas, soil injection/drench and trunk injection are generally preferred over bark/foliar sprays for suppressing pest populations on trees owing to the reduced risk to the applicator and to the environment. Systemic insecticide formulations with different active ingredients [e.g. emamectin benzoate (EB) and imidacloprid (IC)] have been tested for their efficacy against the larvae of the related *A. planipennis* Fairmaire in ash trees.[20–22] These researchers found that a single trunk injection of EB in mid-May or early June can reduce *A. planipennis* larval density over 99% for up to 3 years;[21] however, the efficacy of IC injection or soil drench was less consistent and shorter in duration.[20,22]

In a 2.5 year study, we monitored the impacts of *A. auroguttatus* on coast live oak trees and tested the efficacy of EB and IC trunk injections for control of *A. auroguttatus*. Because many insects prefer to colonize weakened or previously infested trees rather than healthy trees,[23,24] we examined the effect of previous infestation levels on subsequent infestation by *A. auroguttatus* and on the rate of decline of the oaks. We also measured infestation levels as they related to trunk diameter to confirm observations by our group[6,13] that had suggested that larger diameter trees were more likely to be colonized by *A. auroguttatus*. We selected two study sites in the core area of the zone of infestation in San Diego County, California (Fig. 1), and designated coast live oak trees at one site (Deerhorn Valley) for an evaluation of EB and coast live oak trees at the other site (Japatul Valley) for an evaluation of IC.

2 MATERIALS AND METHODS

2.1 Study sites

Two study sites were selected in San Diego County, California. The EB study was located in Deerhorn Valley (32° 40.437′ N, 116° 43.555′ W, 808 m elevation) (Fig. 1), and the IC study was located in Japatul Valley (32° 48.604′ N, 116° 38.997′ W, 1030 m elevation) (Fig. 1). Both sites were primarily oak savannas that intergraded with woodlands along riparian corridors. The other principal tree species was *Q. engelmannii*, which was present at a greater stand density at Japatul Valley than at Deerhorn Valley.

2.2 Pretreatment tree evaluations and insecticide treatments

2.2.1 Deerhorn Valley

On 9–11 March and 7–8 April 2011, 101 coast live oak trees, *Quercus agrifolia*, were inventoried and grouped into four infestation categories [uninfested (40), lightly infested (45), moderately infested (12) and heavily infested (4)]. The categories were based on the numbers of adult *A. auroguttatus* emergence holes on the lower 2 m of the stem: uninfested = 0 holes; lightly infested = 1–9 holes; moderately infested = 10–25 holes; heavily infested = more than 25 holes.[25] The diameter (cm) at breast height (DBH) (ca 1.4 m above the ground) of each experimental tree was also measured and recorded. Trees from each infestation class were assigned at random to control (56) and treatment (45) groups. The DBH of *Q. agrifolia* in Deerhorn Valley ranged from 10 to 110 cm, with most trees falling between 30 and 60 cm (Fig. 2A). When considering the DBH of these trees, there was no significant interaction between the two independent variables (treatment status and infestation category) in Deerhorn Valley ($F_{3,93} = 1.04$, $P > 0.05$). The DBH did not differ between those *Q. agrifolia* selected for insecticide treatment or the control group, nor among the trees in the various infestation categories in April 2011 in Deerhorn Valley (insecticide treatment status: $F_{1,93} = 1.07$, $P > 0.05$; tree infestation level in April 2011: $F_{3,93} = 0.16$, $P > 0.05$).

Emamectin benzoate (TREE-äge®, 4% emamectin benzoate MEC; Syngenta Crop Protection, Greensboro, NC) was injected into the treated groups of trees in the four infestation categories on 7–8 April 2011 by personnel from Syngenta and Arborjet Inc. (Woburn, MA). The application rate used was the medium label rate (i.e. 10 mL of formulated EB per injection hole). The number of injection holes for a given tree was determined by converting the DBH to inches (1 inch = 2.54 cm) and dividing by 2.

2.2.2 Japatul Valley

In spring 2010, 345 *Q. agrifolia* trees in Japatul Valley were inventoried and grouped into infestation categories by using the same method as described for the Deerhorn Valley study. Of the inventoried trees, 147 were uninfested, 193 were lightly infested and five were moderately infested. Heavily infested and dead trees had been removed by the property owner prior to our involvement at the study site. The DBH of *Q. agrifolia* in Japatul Valley ranged from 5 to 160 cm, with most trees falling between 15 and 70 cm (Fig. 2B). Data from all 345 trees were used to explore the relationship between DBH and the cumulative increases in *A. auroguttatus* emergence holes (discussed later).

We intended to select half of the inventoried trees for IC treatment and the other half for assignment to the control group. However, owing to unexpected treatments by the property owner with insecticides other than IC before and after the initiation of our study, 158 trees (52 uninfested and 106 lightly infested) were selected for treatment and 53 trees (32 uninfested, 18 lightly infested and three moderately infested) were assigned to the control group. When considering the DBH of these trees, there was no significant interaction between the two independent variables (treatment status and infestation category) ($F_{1,206} = 0.00$, $P > 0.05$). The DBH did not differ between those *Q. agrifolia* selected for insecticide treatment or the control group ($F_{1,206} = 2.15$, $P > 0.05$). However, the DBH did differ among the trees in the various infestation categories in April 2011 ($F_{2,206} = 0.00$, $P < 0.001$); lightly infested trees were larger in diameter than uninfested trees.

Imidacloprid (IMA-jet, 5.0% imidacloprid; Arborjet, Inc.) was injected into the treated groups of trees in the two infestation categories between 24 March and 25 May 2010, and again between March and May 2011, by a commercial pesticide applicator (Smart Way Landscapes, Descanso, CA). The application rate used was 8 mL of formulated IC per injection hole, as suggested by the label. The number of injection holes for a given tree was determined as in the EB study. Although IC has been applied to trees on an annual basis in past evaluations of ash (*Fraxinus* spp.) and *A. planipennis*,[20–22] in our study the property owner was not willing to tolerate the repeated damage to the trees by injection, so only two applications were made to the treatment group of trees during the course of the study.

2.3 Post-treatment tree evaluations

On 12 October 2011, 18 October 2012 and 22 October 2013, the level of emergence by *A. auroguttatus* and the crown dieback rating for the trees in the two experiments were evaluated. In addition to recording the number of emergence holes from *A. auroguttatus* on each tree, the team also rated the crown dieback of each tree by using the guidelines described in Hishinuma *et al.*,[25] based on percentage of crown leaf loss: 1 = full, healthy crown (0% leaf loss); 2 = minor twig dieback and/or light thinning (10–25% leaf loss); 3 = moderate thinning and twig dieback (25–50% leaf loss); 4 = severe dieback to larger branches (>50% leaf loss); 5 = tree is dead. In October 2011, at both sites, the team re-evaluated the number of emergence holes that had been tallied in April 2011 and verified that the holes were from *A. auroguttatus* [versus from roundheaded woodborers such as *Xylotrechus nauticus* (Mannerheim) (Coleoptera: Cerambycidae)].[10] All *A. auroguttatus* emergence holes were marked with oil-based colored paint pens.

Emergence holes were used in our evaluation because they provide a permanent record of the changes in infestation levels over time. Each hole provides evidence that a single *A. auroguttatus* larva has completed development in the tree and emerged as an adult. *Agrilus auroguttatus* holes are uniquely sized and shaped and, with training, can be distinguished from emergence holes produced by other woodborers in California oaks.[10] In contrast, other life stages are impractical to monitor: adults are rarely observed and the currently recommended detection trap is not very effective;[2] eggs are laid in bark crevices and almost impossible to find; larvae developing within the tree cannot be observed without destructive sampling which would have killed trees and jeopardized our long-term monitoring of insecticide efficacy and tree health. Destructive sampling was also not acceptable at these privately owned sites. By using the number of emergence holes counted before treatment as a base and permanently marking

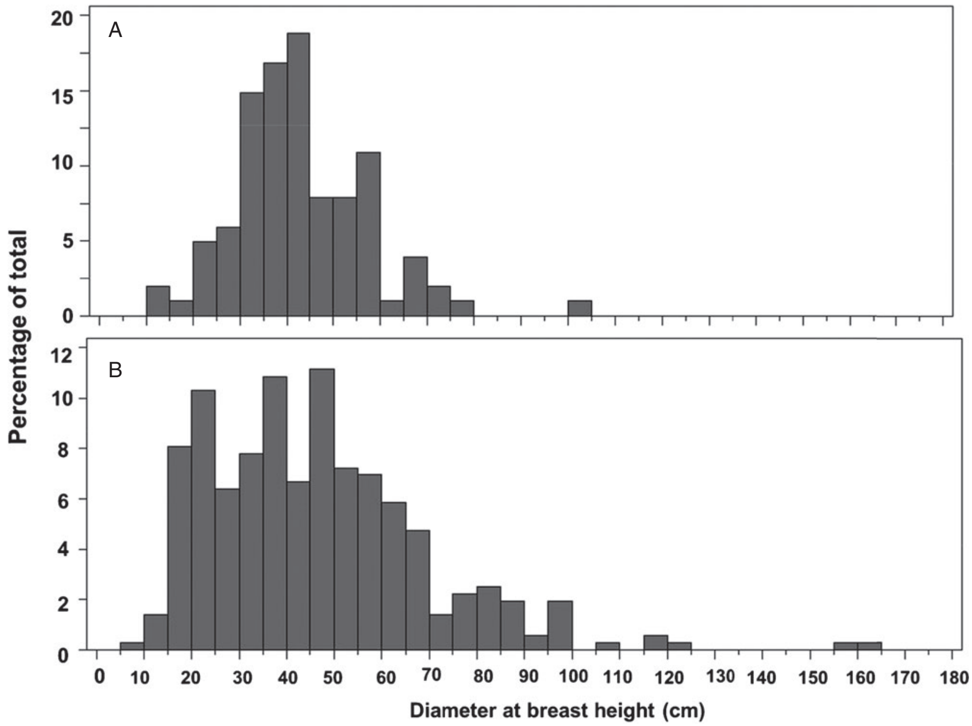

Figure 2. Diameter distribution of *Quercus agrifolia* survey trees in Deerhorn Valley (A, $N = 101$) and Japatul Valley (B, $N = 345$), both located in San Diego County, California.

holes as they were observed in annual evaluations, we determined the increase in the number of holes from each evaluation period to the next and also cumulatively over the entire 2.5 years of the study. Final density (beetles m^{-2} outer bark) was estimated by dividing the total number of emergence holes found on the lower 2 m of each tree stem in October 2013 by surface area ($2 \times 3.14 \times$ DBH m^2) of that trunk section and then compared with similar measurements reported in the literature. The combination of the assessments of emergence hole counts (or density) and crown dieback rating allowed us to standardize and compare the rates of decline of the treated and untreated trees at both sites. The numbers of holes (or their increment or density increment) and the crown dieback rating number (from 1 to 5) became the data that were utilized in the statistical analyses (see below).

2.4 Statistical analyses

Unless otherwise indicated, statistical analyses were conducted by using SAS[26] with a critical threshold for significance $\alpha = 0.05$. DBH data in April 2011 from Deerhorn Valley and Japatul Valley were square-root transformed before being subjected to a two-way ANOVA (PROC GLM). Insecticide treatment status (two levels: treated and untreated control at both sites) and tree infestation class (four levels in Deerhorn Valley: uninfested, lightly infested, moderately infested and heavily infested; two levels in Japatul Valley: uninfested and lightly infested) were the two factors. Normality and variance homogeneity of data were checked with Kolmogorov–Smirnov's *D*-statistic and Levene's test, respectively.

Cumulative increases in *A. auroguttatus* emergence holes from April 2011 to October 2013 in Deerhorn Valley and Japatul Valley were analyzed by generalized linear models with DBH as a covariate (PROC GLIMMIX). Insecticide treatment status and infestation class in April 2011 were two factors in the models. The distribution of cumulative increase in *A. auroguttatus* emergence holes in the Deerhorn Valley dataset was modelled as a negative binomial (conditional on a normally distributed error), and that in Japatul Valley as log normal. The selection of error distributions was based on model fit statistics such as Akaike's information criterion (AIC) (the smaller the better) and Pearson's chi-square.[26] Pearson's chi-square was used to test data dispersion, and a *P*-value of the ratio of Pearson's chi-square to degrees of freedom of the residual greater than 0.05 indicated no under- or overdispersion of the data.

To evaluate effects of insecticide treatment on periodical changes in *A. auroguttatus* emergence holes, annual increases in emergence holes between untreated and EB- or IC-treated trees were analyzed separately by survey period (three survey periods: April 2011–October 2011, October 2011–October 2012 and October 2012–October 2013) by using generalized linear models with DBH as a covariate, as described previously (PROC GLIMMIX).

Another way of measuring the effects of the insecticide treatments was to look at the trend/degree of annual increase in *A. auroguttatus* emergence holes over the three survey periods. Therefore, the medians of the differences in annual increase in emergence holes between two consecutive periods were analyzed by Wilcoxon signed rank tests (*S*-statistics) (PROC UNIVARIATE) separately for each site and each insecticide treatment. A positive *S*-statistic indicates a trend of an increase, whereas a negative *S*-statistic indicates a trend of a decrease.

The rates of decline in *Q. agrifolia* health at the sites were measured as the number of trees remaining or moving through the emergence-hole-based infestation classes or crown dieback rating classes between treatment groups (treated/untreated) or among initial infestation levels (uninfested/lightly infested/moderately infested/heavily infested). These data were analyzed by Pearson's chi-square tests with their corresponding pretreatment percentages.

To delineate any relationship between cumulative increases in *A. auroguttatus* emergence holes and DBH, cumulative increases were regressed against DBH for datasets from both study sites (SigmaPlot 12.0; Systat Software, Inc., Chicago, IL). Data were checked for outliers, and those with studentized residuals greater than 2 were excluded from the regression. Following the method described in Blackburn *et al.*,[27] DBH was divided into 10–20 classes and the greatest value of cumulative increase in emergence holes in each class was used for regression. DBH class width was determined by first subtracting the lowest from the highest value of DBH. This difference was then divided by integers between 10 and 20. A class width of integers was selected. The data were fitted with a Gaussian curve

$$Y = a\,e^{-\frac{x-x_0^2}{2b}}$$

3 RESULTS

3.1 Effects of original infestation class (April 2011) and emamectin benzoate treatment on cumulative increase in *A. auroguttatus* emergence holes and change in infestation class and crown dieback rating between April 2011 and October 2013

3.1.1 Cumulative increase in emergence holes

New *A. auroguttatus* emergence holes were detected in 77 of the 101 study trees surveyed in Deerhorn Valley, which was significantly greater than the number of trees (24) on which no new emergence holes were detected ($\chi^2 = 27.81$, $P < 0.001$). The proportion (41/56 = 73%) of trees on which new emergence holes were detected in the control group was not different from that (36/45 = 80%) in the treatment group ($\chi^2 = 0.00$, $P > 0.05$). Interaction between insecticide treatment status and original infestation class in April 2011 did not affect cumulative increase in *A. auroguttatus* emergence holes in Deerhorn Valley ($F_{3,91} = 0.80$, $P > 0.05$) (Fig. 3A). Insecticide treatment status also had no effect on cumulative increase in *A. auroguttatus* emergence holes ($F_{1,91} = 0.80$, $P > 0.05$) (Fig. 3A). However, the original infestation class in April 2011 significantly affected cumulative increase in *A. auroguttatus* emergence holes ($F_{3,91} = 39.54$. $P < 0.001$) (Fig. 3A). Heavily infested *Q. agrifolia* trees in April 2011 exhibited the greatest cumulative increase in *A. auroguttatus* emergence holes, followed by moderately and lightly infested trees (Fig. 3A). Uninfested trees (whether treated or untreated) exhibited the lowest cumulative increase in *A. auroguttatus* emergence holes (Fig. 3A).

3.1.2 Change in infestation class and crown dieback rating

None of the study trees at Deerhorn Valley died between April 2011 and October 2013. Data on infestation class (one tree missing) and crown dieback rating (six trees missing) were not collected completely in October 2013, so results from only 100 trees were analyzed for infestation class and from only 95 trees for crown dieback rating. In Deerhorn Valley, the infestation class of 56% (56/100) of the *Q. agrifolia* trees increased, whereas 44% (44/100) remained unchanged during the 2.5 year period (Table 1). The infestation class of 57% (32/56) of the 56 untreated trees increased, whereas the infestation class of 55% (24/44) of the 44 EB-treated trees increased (Table 1). The ratio of the two percentages did not differ from the pretreatment ratio of 56% (untreated) to 44% (treated) trees ($\chi^2 = 0.03$, $P > 0.05$). Of the 32 untreated trees that increased in infestation class, at the original assessment point

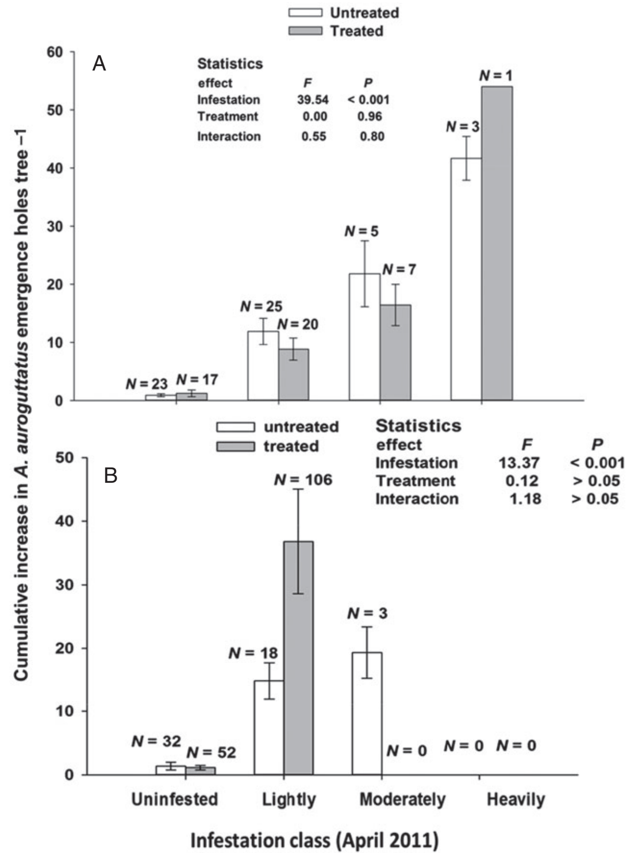

Figure 3. Cumulative increase in *Agrilus auroguttatus* emergence holes (mean ± SE) from April 2011 to October 2013 by original infestation class (in April 2011) and insecticide treatment in Deerhorn Valley (A, emamectin benzoate) and Japatul Valley (B, imidacloprid), San Diego County, California. White bars: untreated trees; gray bars: insecticide-treated trees; sample sizes (number of trees *N*) are provided above the histogram bars.

(April 2011), 34% were uninfested, 53% were lightly infested and 13% were moderately infested (Table 1). The three percentages did not differ from the pretreatment percentages of 43% (uninfested trees), 47% (lightly infested trees) and 9.4% (moderately infested) ($\chi^2 = 1.16$, $P > 0.05$) (Table 1). Of the 24 treated trees that increased in infestation class, 38% were uninfested, 42% were lightly infested and 21% were moderately infested (Table 1). The three percentages did not differ from the pretreatment percentages of 43% (uninfested trees), 47% (lightly infested trees) and 9% (moderately infested) ($\chi^2 = 3.66$, $P > 0.05$) (Table 1). During the 2.5 year EB study, the infestation rate of the 40 originally uninfested trees across the untreated and treated groups was 50% (20/40) (Table 1).

Significantly more trees (treated and untreated; $N = 63$) did not exhibit a change in crown dieback rating when compared with the number that either increased ($N = 17$) or decreased ($N = 15$) in rating ($\chi^2 = 46.57$, $P < 0.001$) (Table 1). Among the 51 untreated trees for which the complete crown dieback dataset was available, the crown dieback rating of 22% increased and that of 12% decreased, which was significantly fewer than the number that remained unchanged (66%) ($\chi^2 = 26.24$, $P < 0.001$). For the 44 treated trees, the crown dieback rating of 14% increased, that of 20% decreased, and that of 66% remained unchanged: the crown dieback rating of significantly more trees remained unchanged ($\chi^2 = 21.32$, $P < 0.001$). The number of untreated and EB-treated trees that changed crown dieback rating or remained unchanged

Table 1. Number of *Quercus agrifolia* trees changing infestation class or crown dieback rating between April 2011 and October 2013 in Deerhorn Valley, San Diego County, California[a]

Category/year 2011 (April)	2013 (October)	Untreated	Treated	Total
Infestation class[b]				
Rating increasing				
Uninfested	Lightly	11	8	19
Uninfested	Moderately	0	1	1
Lightly	Moderately	10	8	18
Lightly	Heavily	7	2	9
Moderately	Heavily	4	5	9
Rating unchanged				
Uninfested	Uninfested	12	8	20
Lightly	Lightly	8	9	17
Moderately	Moderately	1	2	3
Heavily	Heavily	3	1	4
Crown dieback rating[c]				
Rating increasing				
1	2	3	3	6
1	3	1	1	2
2	3	1	0	1
2	4	2	0	2
3	4	4	2	6
Rating decreasing				
4	1	0	1	1
3	2	3	2	5
2	1	3	6	9
Rating unchanged				
1	1	19	20	39
2	2	6	3	9
3	3	4	5	9
4	4	5	1	6

[a] Based on 45 EB-treated and 56 untreated trees; however, data from several trees (one tree for the infestation class and six trees for the crown dieback rating) were incomplete and were not included in the table.
[b] Based on the numbers of emergence holes from adult goldspotted oak borers on the lower 2 m of the stem: uninfested = 0 holes; lightly infested = 1–9 holes; moderately infested = 10–25 holes; heavily infested = more than 25 holes.[25]
[c] Based on the percentage of crown leaf loss: 1 = full, healthy crown (0% leaf loss); 2 = minor twig dieback and/or light thinning (10–25% leaf loss); 3 = moderate thinning and twig dieback (25–50% leaf loss); 4 = severe dieback to larger branches (>50% leaf loss); 5 = tree is dead.[25]

did not differ from the pretreatment ratio of 56 (total untreated trees) to 45 (total treated trees) (Table 1), regardless of the direction of crown dieback rating changes (decreasing infestation class: $\chi^2 = 0.6$, $P > 0.05$; increasing infestation class: $\chi^2 = 1.47$, $P > 0.05$; unchanged: $\chi^2 = 0.40$, $P > 0.05$) (Table 1).

3.2 Effects of original infestation class (April 2011) and imidacloprid treatment on cumulative increase in *A. auroguttatus* emergence holes and change in infestation class and crown dieback rating between April 2011 and October 2013

3.2.1 *Cumulative increase in emergence holes*

New *A. auroguttatus* emergence holes were detected in 138 of the 211 study trees surveyed in Japatul Valley, which was significantly greater than the number of trees (73) on which no new emergence holes were detected ($\chi^2 = 20.02$, $P < 0.001$). The proportion (31/53 = 58%) of trees on which new emergence holes were detected in the control group was not different from that (122/158 = 77%) in the treatment group ($\chi^2 = 1.18$, $P > 0.05$). Interaction between insecticide treatment status and original infestation class in April 2011 did not affect cumulative increase in *A. auroguttatus* emergence holes ($F_{1,146} = 1.18$, $P > 0.05$) (Fig. 3B). Insecticide treatment status also had no effect on cumulative increase in *A. auroguttatus* emergence holes ($F_{1,146} = 0.12$, $P > 0.05$) (Fig. 3B). However, the original infestation class in April 2011 significantly affected cumulative increase in *A. auroguttatus* emergence holes ($F_{2,146} = 13.37$, $P < 0.001$) (Fig. 3B). Lightly and moderately infested trees in April 2011 exhibited greater cumulative increase in *A. auroguttatus* emergence holes than uninfested trees (Fig. 3B). Uninfested trees (whether treated or untreated) exhibited a low cumulative increase in *A. auroguttatus* emergence holes (Fig. 3B).

3.2.2 *Change in infestation class and crown dieback rating*

None of the study trees at Japatul Valley died between April 2011 and October 2013. In Japatul Valley, the infestation class of 53% (112/211) trees increased, whereas that of 47% (99/211) remained unchanged during the 2.5 year period (Table 2). The infestation class of 43% (23/53) of the 53 untreated *Q. agrifolia* trees increased, whereas the infestation class of 56% (89/158) of the 158 IC-treated trees increased (Table 2). The ratio of the two percentages did not differ from the pretreatment ratio of 25% (untreated) to 75% (treated) trees ($\chi^2 = 1.19$, $P > 0.05$). Of the 23 untreated trees that increased in infestation class, 39, 52 and 9% of the trees were originally uninfested, lightly infested and moderately infested respectively (Table 2). The three percentages did not differ from the pretreatment percentages by tree infestation class in April 2011, when 60, 34 and 6% of the survey trees were uninfested, lightly infested and moderately infested respectively ($\chi^2 = 2.33$, $P > 0.05$) (Table 2). Of the 89 treated trees that increased in infestation class, 20 and 80% of the trees were originally uninfested and lightly infested, respectively (Table 2). These percentages differed from the pretreatment percentages of the two tree infestation classes in April 2011, when 33 and 67% of the survey trees were uninfested and lightly infested respectively ($\chi^2 = 4.51$, $P < 0.05$). During the 2.5 year IC study, the infestation rate of the originally uninfested trees across the untreated and treated groups was 32% (27/84) (Table 2).

Significantly more trees (treated and untreated; $N = 113$) did not exhibit a change in crown dieback rating when compared with the number that declined in crown dieback rating ($N = 85$) ($\chi_2 = 3.96$, $P < 0.05$) (Table 2). These trees with a decreasing rating were in turn greater than those with an increasing rating ($N = 8$) ($\chi_2 = 63.75$, $P < 0.001$) (Table 2). For the 50 untreated trees, the crown dieback rating of 2% of the trees increased, and that of 26% of the trees decreased, which was significantly fewer than those that remained unchanged (72%) ($\chi_2 = 37.96$, $P < 0.001$). For the 146 treated trees, the crown dieback rating of 5% of the trees increased, that of 42% of the trees decreased and that of 53% of the trees remained unchanged; the crown dieback rating of significantly more trees remained unchanged ($X^2 = 55.82$, $P < 0.001$). The number of untreated and IC-treated trees that changed crown dieback rating or remained unchanged did not differ from the pretreatment ratio of 53 (total untreated trees) to 158 (total treated trees), regardless of the direction of crown dieback rating changes (decreasing rating: $\chi_2 = 1.89$, $P > 0.05$; increasing rating: $\chi_2 = 0.66$, $P > 0.05$; unchanged: $\chi_2 = 1.68$, $P > 0.05$) (Table 2).

Table 2. Number of *Quercus agrifolia* trees changing infestation class or crown dieback rating between April 2011 and October 2013 in Japatul Valley, San Diego County, California[a]

Category/year				
2011 (April)	2013 (October)	Untreated	Treated	Total
Infestation class[b]				
Rating increasing				
Uninfested	Lightly	7	16	23
Uninfested	Moderately	2	2	4
Lightly	Moderately	8	30	38
Lightly	Heavily	4	41	45
Moderately	Heavily	2	0	2
Rating unchanged				
Uninfested	Uninfested	23	34	57
Lightly	Lightly	6	35	41
Moderately	Moderately	1	0	1
Crown dieback rating[c, d]				
Rating increasing				
1	2	1	2	3
2	3	0	2	2
3	4	0	3	3
Rating decreasing				
4	3	1	11	12
4	2	0	4	4
4	1	1	3	4
3	2	1	14	15
3	1	1	12	13
2	1	9	28	37
Rating unchanged				
1	1	33	59	92
2	2	0	3	3
3	3	3	1	4
4	4	0	14	14

[a] Based on 158 IC-treated and 53 untreated trees; however, crown dieback rating data from five trees were incomplete and were not included in the table.
[b] Based on the numbers of emergence holes from adult goldspotted oak borers on the lower 2 m of the stem: uninfested = 0 holes; lightly infested = 1–9 holes; moderately infested = 10–25 holes; heavily infested = more than 25 holes.[25]
[c] Based on the percentage of crown leaf loss: 1 = full, healthy crown (0% leaf loss); 2 = minor twig dieback and/or light thinning (10–25% leaf loss); 3 = moderate thinning and twig dieback (25–50% leaf loss); 4 = severe dieback to larger branches (>50% leaf loss); 5 = tree is dead.[25]
[d] Crown dieback rating of five trees was incomplete and they were excluded.

3.3 Effects of diameter at breast height on cumulative increase in *A. auroguttatus* emergence holes between April 2011 and October 2013 and total *A. auroguttatus* emergence holes in October 2013

At Deerhorn Valley, trees with a DBH ranging from 20 to 78 cm exhibited the greatest cumulative increase in emergence holes, regardless of insecticide treatment (Fig. 4A). The parameters in the Gaussian curve

$$Y = a\,e^{-\frac{X - X_0^2}{2b}}$$

were $a = 38.50$, $b = 19.37$ and $X_0 = 46.60$ (for all parameters, $P < 0.001$), adj. $R^2 = 0.44$. At Japatul Valley (Fig. 4B), trees with a DBH ranging from 20 to 100 cm exhibited the greatest cumulative increase in emergence holes. The parameters in the Gaussian curve

$$Y = a\,e^{-\frac{X - X_0^2}{2b}}$$

were $a = 110.45$, $b = 36.30$ and $X_0 = 70.34$ (for all parameters $P < 0.01$), adj. $R^2 = 0.21$.

Analysis of data pooled from both study plots revealed that none of the eight trees with DBH < 15 cm in this study was infested in October 2013 (Figs 4C and D). Thus, there is a clear threshold below which coast live oaks might not need to be considered for treatment to prevent colonization by *A. auroguttatus* (Fig. 4D, red line). Furthermore, greater proportions of trees with larger DBH were infested: a little over 50% of the trees with DBH between 15 and 30 cm were infested; over 70% of the trees with DBH between 30 and 105 cm were infested; all six trees with DBH > 105 cm were infested (Fig. 4C). The total number of emergence holes ranged from 0 to approximately 170 per tree (Fig. 4D, one outlier at Japatul Valley with 790 holes not shown), but the majority (93%) of the trees in the survey had less than 60 emergence holes at the end of the survey period.

3.4 Effects of emamectin benzoate and imidacloprid treatment on annual increase in *A. auroguttatus* emergence holes between April 2011 and October 2013

3.4.1 Deerhorn Valley

The annual increase in *A. auroguttatus* emergence holes did not differ between untreated and EB-treated trees, regardless of the survey period (April 2011–October 2011: $F_{1,97} = 0.09$, $P > 0.05$; October 2011–October 2012: $F_{1,97} = 0.12$, $P > 0.05$; October 2012–October 2013: $F_{1,97} = 2.24$, $P > 0.05$) (Fig. 5A). Compared with untreated trees during the April 2011–October 2011 period, EB-treated trees in the same period had an 8% higher annual increase in *A. auroguttatus* emergence holes. However, in the October 2011–October 2012 and October 2012–October 2013 periods, EB-treated trees had 8% and 42% reductions in annual increase in emergence holes, respectively, compared with untreated trees (Fig. 5A). For untreated trees, the medians of the difference in annual increase in *A. auroguttatus* emergence holes between the April 2011–October 2011 and October 2011–October 2012 periods ($S = 200$, $P < 0.001$) and between the April 2011–October 2011 and October 2012–October 2013 periods ($S = -88.5$, $P < 0.05$) were significantly different from zero, and the difference between the October 2011–October 2012 and October 2012–October 2013 periods was not significant ($S = -41$, $P > 0.05$). For EB-treated trees, differences between the survey periods were not significant (between April 2011–October 2011 and October 2011–October 2012: $S = 54$, $P > 0.05$; between October 2011–October 2012 and October 2012–October 2013: $S = -68.5$, $P > 0.05$) (Fig. 5A).

3.4.2 Japatul Valley

The annual increase in *A. auroguttatus* emergence holes did not differ between untreated and IC-treated trees during the first two survey periods (April 2011–October 2011: $F_{1,129} = 3.04$, $P > 0.05$; October 2011–October 2012: $F_{1,118} = 0.19$, $P > 0.05$) (Fig. 5B), but the annual increase in these emergence holes in IC-treated trees was greater than that in untreated trees during the third period (October 2012–October 2013: $F_{1,103} = 4.85$, $P < 0.05$) (Fig. 5B). Compared with untreated trees during the April 2011–October 2011 period, IC-treated trees in the same period had a 390% higher annual increase in *A. auroguttatus* emergence holes. However, in

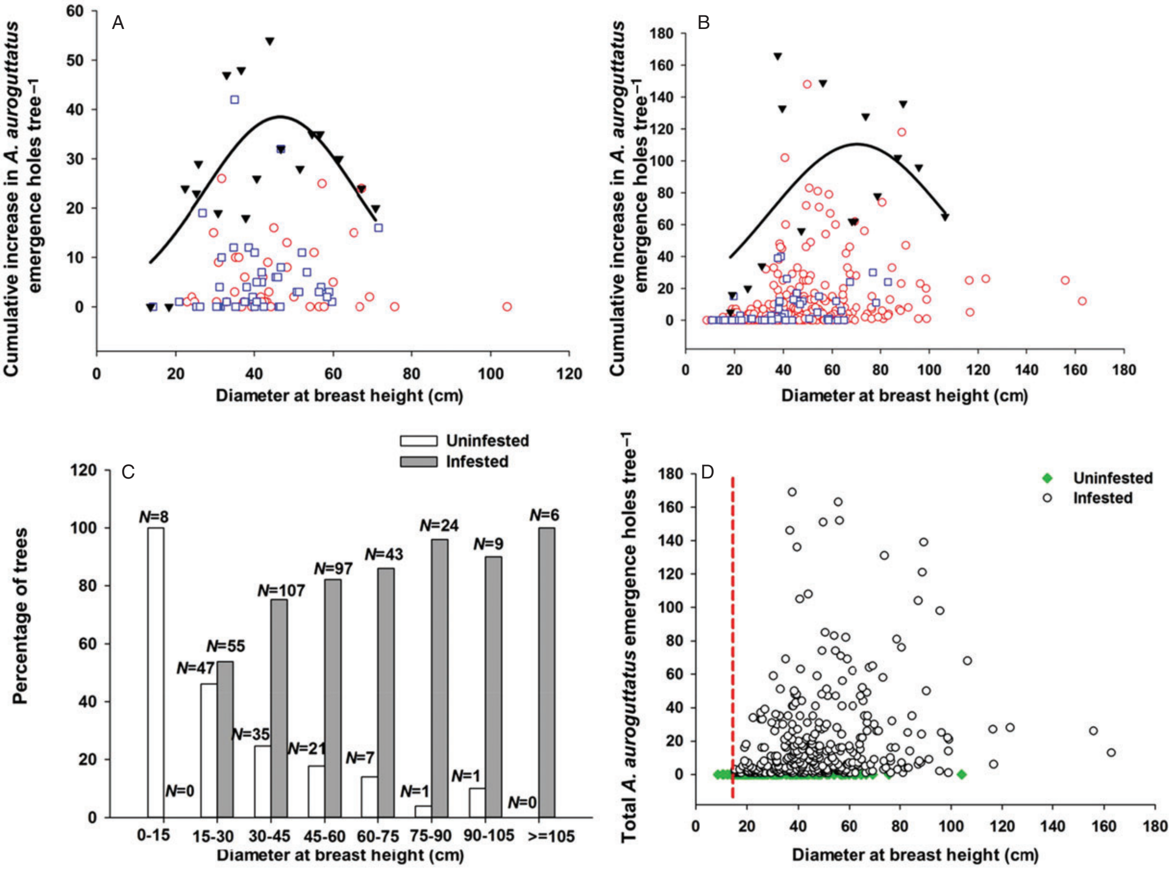

Figure 4. Cumulative increase in and total number of *Agrilus auroguttatus* emergence holes against tree diameter at breast height (DBH): (A) cumulative increase in *A. auroguttatus* emergence holes from April 2011 to October 2013 against DBH in Deerhorn Valley ($N = 101$); (B) cumulative increase in *A. auroguttatus* emergence holes from April 2011 to October 2013 against DBH in Japatul Valley ($N = 345$); (C) percentage of *Q. agrifolia* trees in Deerhorn Valley and Japatul Valley infested by *A. auroguttatus* in October 2013 by DBH classes ($N = 446$); (D) total *A. auroguttatus* emergence holes pooled from both Deerhorn and Japatul Valley datasets against DBH ($N = 445$). Red open circles, blue open squares and black solid triangules in (A) and (B) denote insecticide-treated trees, untreated trees and points used to fit the regression curves respectively. Black open and green solid circles in (D) denote uninfested and infested trees respectively. The red line in (D) indicates a DBH of 15 cm, below which emergence does not appear to occur. Tree 28 (DBH = 60.20 cm) in Japatul Valley had 790 new emergence holes (outlier) and was not included in the graph (D).

the October 2011–October 2012 and October 2012–October 2013 periods, IC-treated trees only had 137 and 235% greater annual increases in emergence holes, respectively, when compared with untreated trees (Fig. 5B). For untreated trees, the median of the difference in annual increase in *A. auroguttatus* emergence holes between the April 2011–October 2011 and October 2011–October 2012 periods was not significantly different from zero ($S = -14.5$, $P > 0.05$), and the difference between October 2011–October 2012 and October 2012–October 2013 was also not significant ($S = -54.5$, $P > 0.05$). For IC-treated trees, both differences in annual increase, one between the April 2011–October 2011 and October 2011–October 2012 periods and the other between the October 2011–October 2012 and October 2012–October 2013 periods, were significant (between April 2011–October 2011 and October 2011–October 2012: $S = -2008$, $P < 0.001$; between October 2011–October 2012 and October 2012–October 2013: $S = -742.5$, $P < 0.05$) (Fig. 5B).

4 DISCUSSION

4.1 Infestation class and *A. auroguttatus* emergence holes

Greater cumulative increases in *A. auroguttatus* emergence holes were observed in infested trees than in uninfested trees at both study sites (Fig. 3), regardless of insecticide treatment. This suggests that females may be ovipositing more frequently on infested trees and/or larvae may be better able to complete their development in infested trees.

Greater rates of colonization of infested trees have also been documented in *A. planipennis*.[24] The reason for greater rates of colonization of infested trees by *Agrilus* spp. is likely complex. The underlying mechanism(s) may reflect the benefits of aggregation for this insect group. Insects[23,24,28] and many other animals[29−31] aggregate for many reasons. Bark beetles (Coleoptera: Scolytidae)[32] use pheromones to aggregate and overwhelm host plant defenses.[23,28,33] Spiders aggregate and increase the frequency/intensity of vibrations in the colony web to reduce predation risks from predatory wasps.[31] If aggregation is a key factor in colonization of oaks by *A. auroguttatus*, effective management of the pest might focus on removal of moderately to severely infested trees[13] and insecticide application to lightly infested trees. This management activity may require more effective early detection techniques than are available presently.[2]

4.2 DBH and *A. auroguttatus* emergence holes

As first reported by Coleman *et al.*,[6] we have confirmed in the core area of the infestation that larger coast live oak trees are more likely

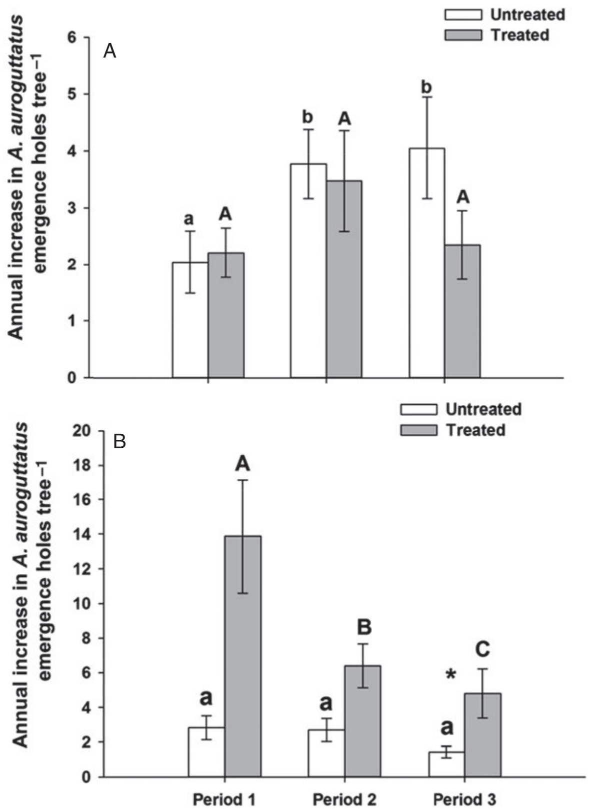

Figure 5. Annual increase in *Agrilus auroguttatus* emergence holes (mean ± SE) during three survey periods in Deerhorn Valley (A) and Japatul Valley (B), San Diego County, California. Period 1: April 2011–October 2011; period 2: October 2011–October 2012; period 3: October 2012–October 2013. An asterisk (*) above a cluster of bars indicates a significant difference between treated and untreated trees within a survey period (PROC GLIMMIX with DBH as a covariate); white bars topped with different lower-case letters indicate significant differences among survey periods for untreated trees (Wilcoxon signed rank tests, PROC UNIVARIATE); gray bars topped with different upper-case letters indicate significant differences among survey periods for treated trees (Wilcoxon signed rank tests, PROC UNIVARIATE). Deerhorn Valley: $N_{\text{untreated}}$ during each period was 56; $N_{\text{emamectin-benzoate-treated}}$ during the three periods was 45, 45 and 44 respectively; Japatul Valley: $N_{\text{untreated}}$ during the three periods was 53; $N_{\text{imidacloprid-treated}}$ during the three periods was 158.

to be colonized by *A. auroguttatus*. Oak trees with DBH > 13 cm are generally susceptible to *A. auroguttatus* infestation;[6] none of the eight trees in our study with DBH < 15 cm was infested (Figs 4C and D). Moreover, *Q. agrifolia* with DBH > 30 cm were more frequently infested with *A. auroguttatus* than smaller trees (the present study and Coleman *et al.*[6]), and almost all trees with DBH > 80 cm were infested (the present study and Coleman *et al.*[2,6]) (Fig. 4C). At newly infested sites on the periphery of the zone of infestation, *Q. agrifolia* in the 11–20 cm diameter class were rarely infested, whereas greater proportions of *Q. agrifolia* in the larger diameter classes were infested and, in particular, brood trees were observed in the larger diameter classes (>41 cm DBH).[13] At both Deerhorn Valley and Japatul Valley, the greatest cumulative increase in emergence holes was observed on trees with an intermediate DBH (between 20 and 41 cm) (Figs 4A and B).

4.3 Emergence hole density and rate of *A. auroguttatus* infestation and *Q. agrifolia* decline

Phloem- and xylem-boring insects in the genus *Agrilus* are among the most destructive forest insects because of their larval feeding activity in and adult emergence from the main stem of trees.[34] Emergence hole densities of *Agrilus* spp. on dead host hardwood trees have varied in past studies (Table 3). For instance, the mean density of *A. planipennis* emergence holes on dead green ash, *Fraxinus pennsylvanica* Marshall, and white ash, *F. americana* L., was 89 beetles m^{-2} (range 17–170),[35] whereas the mean density of *A. auroguttatus* on dead *Q. agrifolia* trees was 111 beetles m^{-2} (range 11–556).[7] The mean densities of *A. auroguttatus* emergence holes on the live *Q. agrifolia* in the two sites in this study were much lower (6 beetles m^{-2}), which is comparable with previous assessments of emergence density of *A. auroguttatus* from live *Q. agrifolia* (Table 3). The maximum density of *A. auroguttatus* emergence holes on *Q. agrifolia* in Japatul Valley was more than triple that on trees in Deerhorn Valley (Table 3). In spite of these occasional high emergence densities, no survey trees died during the 2.5 year study period at either site. However, ca 13% of red oak trees have been killed by *A. auroguttatus* in other infested oak woodlands in San Diego County, and ca 2% of red oak trees have been killed by this beetle in its native range in Arizona.[6] In a survey of *A. auroguttatus* emergence densities and impact on oaks, Haavik *et al.*[13] reported a greater proportion of dead *Q. agrifolia* (9/722) than we measured in this study. Five of those 9 trees were killed by *A. auroguttatus*.

The absence of tree mortality in spite of the high rate of *A. auroguttatus* emergence and declining crowns on some trees in the present study might indicate that some *Q. agrifolia* trees in the population have greater intrinsic tolerance of this pest. Other invasive insects and pathogens have been observed to kill most, but not all, hosts as the pests have established and then expanded their ranges. This host tolerance among hardwoods has been noted with *Fraxinus* and *A. planipennis*,[36] Dutch elm disease,[37] sudden oak death,[38,39] and thousand cankers disease of walnut.[40] Alternatively, the survival of *Q. agrifolia* under high levels of *A. auroguttatus* infestation might be attributable to more aggressive management in private ranches compared with natural forests. For example, prior to the experiment, more than 50 previously killed *Q. agrifolia* had been removed by the homeowner at the Japatul Valley site.[6] Lastly, precipitation patterns at the study sites during the study period might also have contributed to the survival of *Q. agrifolia* during our 2.5 years of observation. Although it was not measured locally at the two study sites, the mean annual precipitation from 2010 to 2012 at Otay Lake, California (approximately 60 km southwest of the study sites), was 22.69 cm, which was more than double that from 2004 to 2009 (12.10 cm) (data from weather station No. 147 were obtained from the website of the California Irrigation Management Information System, Department of Water Resources: http://www.cimis.water.ca.gov/, last accessed 13 June 2014). The precipitation in 2013 was zero. In contrast, the air and soil temperatures between the 2004–2009 and 2010–2013 periods from the weather station were both very similar.

The infestation class ratings of 44% (Deerhorn Valley) or 47% (Japatul Valley) of the *Q. agrifolia* trees remained unchanged (Tables 1 and 2). Furthermore, the crown dieback ratings of most trees in both sites remained unchanged, regardless of insecticide treatment (Tables 1 and 2). Taken together, these results and the absence of tree mortality in the study might indicate that the *Q. agrifolia* trees at this stage of the local infestation were able to tolerate some degree of colonization by *A. auroguttatus* and that the oak decline and mortality in response to *A. auroguttatus* are relatively gradual. Admittedly, our study was of short duration and may not have lasted long enough to capture these changes in

Table 3. Comparison of mean densities (range) of *Agrilus* spp. emergence holes on dead and live host trees reported in the literature and from this study

Species	Host	Sample size (*N*)	Mean density (m^{-2} of bark) [mean (range)]	Reference
Dead trees				
A. anxius	*Betula pendula*	n/a[a]	232 (n/a)	Akers and Nielsen[52]
A. auroguttatus	*Quercus agrifolia*	99	111 (11–556)	Haavik *et al.*[7]
	Q. kelloggii	91	56 (0–178)	Haavik *et al.*[7]
	Q. emoryi	40	11 (0–78)	Haavik *et al.*[7]
	Q. hypoleucoides	33	1 (0–133)	Haavik *et al.*[7]
A. planipennis	*Fraxinus pennsylvanica* and *F. americana*	71	89 (17–170)	McCullough and Siegert[35]
Live trees				
A. auroguttatus	*Q. agrifolia*	345	6 (0–210)[b]	Japatul Valley, this study
	Q. agrifolia	101	6 (0–63)[b]	Deerhorn Valley, this study
	Q. agrifolia	144	4 (0–102)	Coleman *et al.*[2]
	Q. agrifolia	722	1.4 (0.6–2.5)[c]	Haavik *et al.*[13]

[a] Not available.
[b] Density based on survey in October 2013.
[c] Density represents the grand mean of the mean densities from six nascently infested sites in San Diego County, California: Potrero (93 trees), Mataguay (175 trees), Marian Bear (53 trees), Loveland (169 trees), La Posta (161 trees) and Boulder Creek (71 trees); range denotes the minimum and maximum mean densities of the six sites.

the landscape. However, Haavik *et al.*[13] also reported generally low rates of appearance of symptoms of *A. auroguttatus* infestation (emergence hole density, woodpecker activity and bark staining), as well as tree mortality (5 out of 722 trees), at six survey sites on the periphery of the zone of infestation in San Diego County. Lynch *et al.*[41] also reported low levels of oak mortality in the same general area.

In some instances, crown dieback rating decreased during the survey period (Tables 1 and 2). In other words, the crowns of some survey trees improved during the course of the study. This is a bit counterintuitive, but in some cases we hypothesize that, either because of improving water relations or because of the impact of the systemic insecticides, trees improved in crown dieback rating (typically '2 s' became '1 s' or '3 s' became '2 s') during the course of the multiyear survey. Some of these trees may even have accumulated a few more emergence holes, but overall the crowns took on a greener appearance and may have retained more foliage. This appeared to be especially evident with the IC-treated trees (Table 2).

4.4 Emamectin benzoate treatment

EB treatment did not significantly affect the cumulative increases in *A. auroguttatus* emergence holes in *Q. agrifolia* at Deerhorn Valley between April 2011 and October 2013 (Fig. 3A). EB treatment also did not significantly affect the increases in *A. auroguttatus* emergence holes in *Q. agrifolia* during the first survey period (April 2011 to October 2011) (Fig. 5A). This latter result is not surprising because the beetles emerging from these holes likely had completed development as larvae and had moved to the outer phloem before the insecticide treatment in April 2011. Thus, these prepupae would have not had the opportunity to ingest the active ingredient present hypothetically more deeply in the phloem. However, during the two subsequent periods of assessment, EB treatment did appear to reduce the pest's success at completing development in treated trees, although these direct comparisons were not significant (Fig. 5A). The annual increase in *A. auroguttatus* emergence holes between October 2011 and October 2012 in the untreated trees was significantly greater than that during the previous survey period (April 2011 to October 2011) (Fig. 5A). However, this was not the case for treated trees: the annual increase in *A. auroguttatus* emergence holes between October 2011 and October 2012 did not differ from that during the previous survey period (April 2011–October 2011) (Fig. 5A). The new emergence holes observed in October 2012 would have been made by *A. auroguttatus* that had developed from eggs oviposited from June to September 2011[10] and subsequent to EB injection (April 2011). Assuming identical factors determining oviposition preference of *A. auroguttatus* females on treated and untreated trees (other than the treatment), this implies that a smaller proportion of *A. auroguttatus* larvae survived to the adult stage. Compared with the untreated control, trunk injection of EB significantly reduced *A. planipennis* larval density on *Fraxinus* sp. trees.[42] The annual increase in emergence holes between October 2012 and October 2013 in untreated trees remained high (the mean was close to that during the previous period) (Fig. 5A), whereas the annual increase in emergence holes in EB-treated trees declined relative to that during the previous period (Fig. 5A), perhaps indicating a multiyear effect of EB.

The exact mechanism of persistence of EB in trees is unknown. EB protects Japanese black pine, *Pinus thunbergii* Palatore, and Japanese red pine, *P. densiflora* Siebold & Zucarini, from pine wood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer Nickle), damage by inhibition of propagation for at least 3 years.[43] It also protects green ash, *F. pennsylvanica*, trees from *A. planipennis* infestation for 3 years.[21] Likewise, following a single treatment, EB was effective in protection of ponderosa pine, *Pinus ponderosa* Douglas *ex* C. Lawson, from western pine beetle, *Dendroctonus brevicomis* LeConte (Coleoptera: Scolytidae), during the third year after treatment.[44]

The mode of action of EB on *A. auroguttatus* remains to be determined. In green ash trees, it has been suggested that EB might act as an oviposition repellent or be toxic to first-instar larvae of *A. planipennis*: when destructively sampled, treated ash

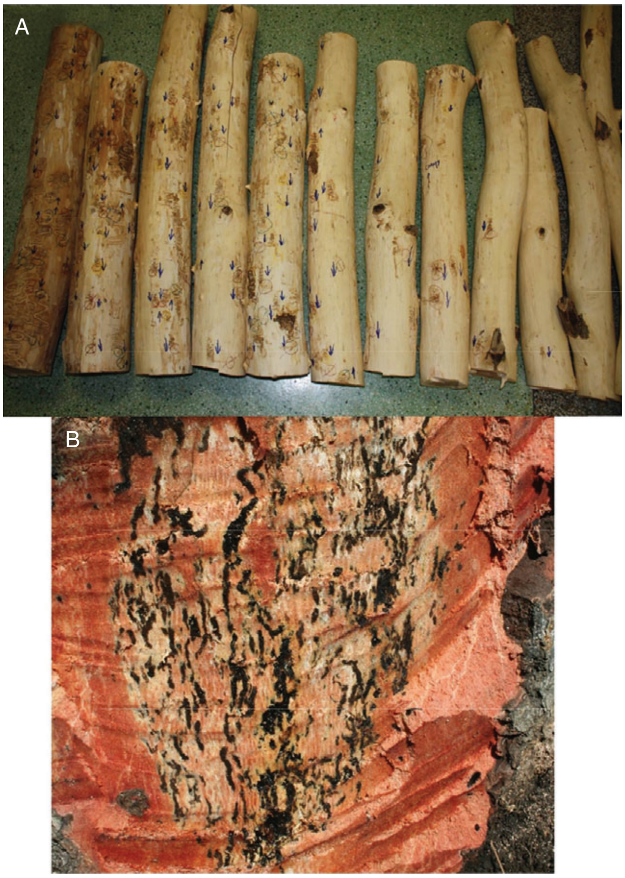

Figure 6. Main stems of green ash *Fraxinus pennsylvanica* (A) and coast live oak *Quercus agrifolia* (B), showing the ease/difficulty of peeling bark (photo A was taken by Chen Y; photo B was taken by Coleman TW).

trees were found to have a lower density of larvae[42] or no larvae[21] within wood compared with untreated trees. EB is also highly toxic to *A. planipennis* adults in leaf-feeding bioassays.[42] Because female *Agrilus* do not pierce the outer bark with their mouthparts or ovipositor, it seems that EB (or any xylem- or phloem-mobile systemic insecticide) is less likely to act as an oviposition deterrent and more likely to function as a larvicide in the phloem and as an adulticide, i.e. killing adults that feed on leaves before they oviposit. Compared with the high efficacy of EB on *A. planipennis* in ash trees, the marginally suppressive effect of EB on the appearance of *A. auroguttatus* emergence holes on oak trees might have been a consequence of a difference in the physical structure of oaks: the phloem of oak trees is much thicker than that of ash trees (Fig. 6). Alternatively, it might have been attributable to the relatively low precipitation at the site, which reduces the translocation of EB within an oak tree.

4.5 Imidacloprid treatment

IC treatment did not significantly affect the cumulative increases in *A. auroguttatus* emergence holes in *Q. agrifolia* at Japatul Valley over the 2.5 year period between April 2011 and October 2013 (Fig. 3B). However, the annual increase in *A. auroguttatus* emergence holes in IC-treated *Q. agrifolia* between October 2012 and October 2013 was significantly greater than that of untreated trees (Fig. 5B). Together with the observation that IC-treated trees had 390 and 137% higher annual increases in *A. auroguttatus* emergence holes in the April 2011–October 2011 and October 2011–October 2012 periods than untreated trees in the same period, respectively (Fig. 5B), this might suggest that IC injection increased *A. auroguttatus* oviposition rate, larval survival from parasitism/predation and/or larval developmental capacity. On the other hand, the significant reduction in annual increase in emergence holes on treated trees over the three survey periods, compared with no reduction in annual increase in emergence holes on untreated trees over the survey periods, indicates a negative impact of the IC treatment on *A. auroguttatus*. The reasons for these conflicting findings are unclear, but they may reflect a lack of persistence of IC in the 2012 and 2013 survey periods, where treatments had last been applied to trees more than 17 and 29 months previously, respectively.

IC is transported primarily upwards from the injection points in the xylem to leaves in ash trees (*Fraxinus* spp.),[45–47] and little is found in the outer bark and phloem tissue after 2 years.[46] Mota-Sanchez *et al.*[46] found that IC concentrations in leaves continually increased over 3 months after injection, but 9 months later they were reduced by nearly 90%. Provided that it acts the same way in oak trees, IC's minor suppressive effect against *A. auroguttatus* might be attributable to effects on larvae or adults. IC injected into poplar trees, *Populus* spp., reportedly kills adult *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) or potentially other life stages.[48]

4.6 Challenges when conducting research related to insecticidal treatments for *A. auroguttatus*

Although the present study provided some valuable information on the efficacy of EB and IC on *A. auroguttatus*, it also revealed the challenges faced when evaluating insecticides for controlling this pest. Firstly, the slow rates of tree mortality and potentially declining natural *A. auroguttatus* populations (Fig. 5B) make it difficult to evaluate the efficacy of insecticides in a short timeframe. In a similar study, a low population density and slow attack rate by *D. brevicomis* hindered the evaluation of efficacy of EB and other insecticides to protect *P. ponderosa*.[44] Secondly, the cryptic biology of *A. auroguttatus* larvae and the preference of *A. auroguttatus* for attacking lower main stems of large *Q. agrifolia* trees[49] make it difficult logistically to evaluate the efficacy of insecticides to kill larvae. The trees are simply too large to manipulate. Although *A. planipennis* exhibits the same larval feeding biology, it is more convenient to study because it attacks and develops in ash branches as small as 2 cm in diameter, making dissection of infested branches feasible following insecticide assays.[24] In contrast, *A. auroguttatus* mainly attacks the trunks of oak trees with DBH > 20 cm (Fig. 4).[6] Thirdly, the physical structure of oak trees poses great difficulty for insecticide assays with larvae. Insecticide assays with *A. planipennis* typically have involved peeling the relatively thin and loose ash bark (i.e. dead outer bark and live inner bark).[21,22,42] Compared with ash trees, the bark of oak trees, in particular *Q. agrifolia* used in the present study, is very thick and is attached tightly to the phloem/xylem (private observation) (Fig. 6), making it impossible to observe larvae developing beneath the bark. Finally, the absence of a strong attractant for *A. auroguttatus* limits our ability to manipulate attack rates on experimental trees, which has been a useful technique with other subcortical insects. For instance, in studies of the efficacy of insecticides (including EB) for the control of conifer bark beetles, aggregation pheromones are used routinely to challenge the experimental trees.[44,50,51]

4.7 Factors to consider when implementing *A. auroguttatus* management programs

These results provide insights that may be helpful in building management programs for *A. auroguttatus*. Because no *Q. agrifolia* trees died and their health declined slowly during the 2.5 year study, there may be a window for implementing tree protection measures after infestations are discovered but before trees are killed. Management efforts should focus on medium to large trees, as *A. auroguttatus* prefers trees with DBH > 15 – 30 cm. Small trees likely do not need to be treated; however, no research has been carried out to evaluate sites in the core areas (i.e. where beetle population density is high) that only have small trees. This would provide a rigorous test of the susceptibility of small-diameter trees. The findings that previously infested trees are more prone to further colonization suggests that as part of an IPM program it may be advisable to remove them if they are heavily infested. The two systemic insecticides tested in the study had minor suppressive effects, but they did not control *A. auroguttatus*. At present, preventing movement of infested wood into new areas remains the key tool available for reducing spread of this invasive pest.

Future studies related to management of *A. auroguttatus* with insecticides might include the evaluation of contact sprays, as well as further work with systemic insecticides to refine the timing, dosage and return interval for the treatments. We intend to retreat the trees in our two studies at approximately 3.5 years with the systemic insecticides, and to monitor the sites for a longer term in anticipation of eventual tree mortality in the control groups. These future insecticide trials might also include detection of temporal and spatial patterns of insecticides within oak trees by using an enzyme-linked immunosorbent assay (ELISA) for the active ingredient, feeding assays with *A. auroguttatus* adults and larvae, monitoring of the level of *A. auroguttatus* larval mortality by felling and dissecting intermediate-sized infested trees and determination of oviposition preference of adult *A. auroguttatus* on insecticide-treated and untreated trees.

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